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(54) Title: METHOD AND APPARATUS FOR VIEWING A CULTURE MEDIUM HAVING VISIBLE BACTERIAL COLONIES

(57) Abstract: The invention is a method and an apparatus for viewing a culture medium (14) having visible bacterial colonies (24). An apparatus in accordance with the invention includes a light source (10), which provides incident light on the culture medium having the visible bacterial colonies; a polarizer (12), which polarizes the incident light; an image-producing device (22) having a field of view, including the culture medium, which provides an image of the field of view; and an analyzer (16), which at least partially blocks reflected polarized light from being transmitted from the bacterial colonies and culture medium to the image-producing device and which passes back-scattered light transmitted from the bacterial colonies and the culture medium to the images-producing device to cause the image-producing device to produce an image of the back-scattered light of the visible bacterial colonies and the culture medium.

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METHOD AND APPARATUS FOR VIEWING A CULTURE MEDIUM HAVING VISIBLE BACTERIAL COLONIES

TECHNICAL FIELD

The present invention relates to methods and apparatus for viewing culture media having visible bacterial colonies.

BACKGROUND ART

Conventional analysis of samples that contain bacteria whose numbers are to be counted utilize pour plates or spread plates containing a culture medium that is inoculated with the sample. A sample containing bacteria is applied either across the top surface of a spread plate or throughout the volume of a pour plate to innoculate the plate for bacterial growth. After incubation, visible bacterial colonies, which have an appearance typically in the form of a raised pattern of different diameters, may be seen on the top surface of the plates. The degree of contamination of the sample with bacteria may be determined by counting the number of visible colonies that have appeared on the culture medium after incubation. The higher the number of colonies counted, the higher the degree of contamination.

Originally, "colony counting" was a manual operation that required a person to count the number of visible bacterial colonies on the culture medium after incubation. Many years ago, the assignee of the present invention developed a laser colony counter that scanned the incubated culture medium with a laser beam to count the number of visible colonies.

Synoptics of Great Britain sells a colony counter, marketed as the PROTOCOL™, which uses a video camera. A circular light source illuminates the culture medium having visible colonies to be counted. The video camera produces a video image of the culture medium containing the visible colonies, which is processed to produce a colony count.

The objective of colony counting is to obtain the most accurate count of visible colonies on the incubated plate with the least amount of human effort, i.e. a totally automated and accurate colony counting process.

The problem that is associated with automated colony counters is that these devices lack sufficient intelligence to make counting judgments that would routinely

The problem that is associated with automated colony counters is that these devices lack sufficient intelligence to make counting judgments that would routinely be made by a counting process implemented by persons. Over the years, software used in automated counting processes has improved but, nonetheless, problems still exist with automated colony counters because the operation often counts more colonies as being present than are actually present on the surface of the culture medium.

Another use of culture media is to test the effectiveness of compounds (antibiotics) in inhibiting the growth of bacteria in the culture medium. One such process utilizes so-called "zones of inhibition", which are areas on the culture medium wherein an inhibitory compound is of sufficient concentration to prevent or slow the growth of bacteria. In typical practice, zones are created by seeding an agar culture medium with bacteria, placing antibiotic-impregnated disks at specified locations on the medium and allowing the bacteria to grow. After incubation, a lawn of bacteria covers the plate except in areas where the antibiotic has diffused into the culture medium to a toxic level. This area of clearing is called the "zone of inhibition". The zone diameters are measured to produce a direct correspondence of the susceptibility of the bacteria to the compound used. Zones of inhibition may exhibit abrupt interfaces from growth to no-growth.

Another process which uses culture mediums is the "spiral gradient endpoint" or SGE method for determining the minimum inhibitory concentration (MIC) of an antibiotic and for evaluating the resistance development in bacteria to an antibiotic. A gradient of an antibiotic is formed on a plate using what is known as a "spiral plater", which is a well-known apparatus sold by the Assignee of the present invention. A spiral plater lays down a gradient of the antibiotic being tested in an Archimedes spiral on the culture medium plate. Bacterial suspensions are swabbed in radial spokes on the plate. After incubation, the radial position of the endpoint of growth is used to calculate the MIC since the programmed gradient of deposition of the Archimedes spiral is known at the radial position of the end point of growth.

United States Patent 2,318,705 discloses a metallographic filtering system which uses a pair of orthogonally disposed polarizing screens to remove reflected light from the eyepiece of a microscope used for the examination of opaque specimens illuminated by normal illumination.

United States Patent 2,947,212 discloses a method of detecting surface conditions on sheet metal that uses a pair of polarizing members to form an image of the sheet metal with only polarized light so as to reject extraneous light rays.

United States Patent 5,442,489 discloses a video imaging device providing a magnified image. The device utilizes both polarized light and non-polarized light to selectively permit viewing of an image from reflected and non-reflected surface light.

United States Patent 5,742,392 discloses an apparatus that selectively permits viewing of an image that is reflected from the surface or from the subsurface of the object of inspection. This apparatus is described as being used to form images of polarized light reflected from a surface of the object of inspection and non-polarized light from the subsurface thereof.

DISCLOSURE OF THE INVENTION

The present invention is an apparatus and method for viewing a culture medium having visible bacterial colonies, which produces an improved image of visible bacterial colonies present on the culture medium to facilitate automated colony counting. The improved image makes it possible for software, which is not part of the present invention and which is to be marketed by the Assignee of the present invention, to produce a more accurate colony count.

The present invention is based upon the discovery that a degraded optical image of a culture medium having visible bacterial colonies is produced by illumination of the bacterial colonies and media by a direct light source. It has been discovered that a better visible image of bacterial colonies on the culture medium and a more accurate colony count resultant from the aforementioned software is obtained when the light source is filtered by a polarizer-analyzer system. The bacterial colonies and the culture medium are illuminated with polarized light which is then reflected and back-scattered to an analyzer having a polarization axis orthogonal to a polarization axis of the polarizer. The analyzer blocks polarized light reflected from the surface of the bacterial colonies and the surrounding areas of the culture medium without visible bacterial colonies and passes non-polarized back-scattered light from the bacterial colonies and the surrounding culture medium to the image-producing device. As a consequence of the image of the visible colonies and the culture medium being formed principally from back-

scattered light which is not polarized, a clear image of the bacterial colonies without reflections is formed. Such reflections have been discovered as being the cause of the poor performance and reduced accuracy of image scanners in the prior art.

An apparatus for viewing a culture medium having visible bacterial colonies in accordance with the invention includes a light source, which provides incident light on the culture medium having the visible bacterial colonies; a polarizer, which polarizes the incident light; an image producing device having a field of view, including the culture medium, which provides an image of the field of view; and an analyzer, which at least partially blocks reflected polarized light from being transmitted from the bacterial colonies and culture medium to the image-producing device and which passes back-scattered light transmitted from the bacterial colonies and the culture medium to the image-producing device to cause the image-producing device to produce an image of the back-scattered light of the visible bacterial colonies and the culture medium. The polarizer may be disposed between the culture medium and the light source; and the analyzer may be disposed in the field of view between the image-producing device and the culture medium. The reflected light may be from individual bacterial colonies or a transition of an area of growth of bacterial colonies to an area of no growth of bacterial colonies. The image-producing device may be a camera and in a preferred embodiment is a video camera. The polarizer and analyzer may each linearly polarize light and the analyzer may have a polarization axis which is orthogonal to a polarization axis of the polarizer.

A method of viewing a culture medium having visible bacterial colonies with an apparatus having a light source, a polarizer, an image-producing device, and an analyzer, in accordance with the invention, includes illuminating the culture medium with light from the light source which passes through the polarizer before illuminating the culture medium with polarized light; transmitting reflected and back-scattered light from the culture medium and the bacterial colonies on the culture medium to the analyzer, which blocks at least part of the polarized light from being transmitted to the image-producing device and transmits the back scattered light to the image-producing device; and producing an image with the back-scattered light of the bacterial colonies and the culture medium with the image-producing device. The reflected and back-scattered light may be transmitted from individual bacterial colonies or from a transition of an area of

growth of bacterial colonies to an area of no growth of bacterial colonies. The polarizer and analyzer each may linearly polarize light; and the analyzer may have a polarization axis which is orthogonal to a polarization axis of the polarizer.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the present invention used for the counting of individual microbial colonies.

Fig. 2 illustrates the present invention used for the analysis of a zone of inhibition.

Fig. 3 illustrates the present invention used for the analysis of SGE streak.

Fig. 4 is a photograph of an actual image of a culture medium having visible bacterial colonies produced by the present invention with the polarization axis of the analyzer parallel to the polarization axis of the polarizer.

Fig. 5 is a photograph of an actual image of the same culture medium of Fig. 4 with the polarization axis of the analyzer orthogonal to the polarization axis of the polarizer.

Fig. 6 is a photograph of the same culture medium of Fig. 4 with bacterial colony identifying indicia being applied by software that processes the video output produced by the present invention.

Fig. 7 is a photograph of the same culture medium of Fig. 5 with bacterial colony identifying indicia being applied by the same software used to produce the image of Fig. 6.

Like reference numerals identify like parts throughout the drawings.

BEST MODE FOR CARRYING OUT THE INVENTION

The embodiments of the present invention in Figs. 1-3 are all structurally identical and differ only in the particular characteristics of the incubated cultural media as described below, which respectively are an image of individual bacterial colonies, a zone of inhibition, and a SGE streak.

The component parts of the apparatus of Figs. 1-3 are a light source 10 in the form of a circular bulb which may be fluorescent; a polarizer 12, which produces plane

polarized light from the light source 10, which passes through the polarizer to illuminate, a culture medium 14, which may be a petri plate or other media that is illuminated with polarized light passing through the polarizer 12; an analyzer 16, which is a polarizer having an axis of polarization orthogonal to the axis of polarization to the polarizer 12; a lens 18, which forms an image of a field of view of light passing from the culture medium 14 through aperture 20 of the polarizer 12; and a video camera 22, which produces a video image of the culture medium including any visible bacterial colonies thereon as, for example, illustrated in Figs 4-7.

The operation of the aforementioned apparatus is to produce an image of individual bacterial colonies or areas of transition from bacterial growth to no bacterial growth from the non-polarized back-scattered light transmitted from the culture medium 14, passed through the analyzer 16 and to the lens 18. Polarized light produced by the polarizer 12 is reflected from bacterial colonies or the surface of the culture medium 14, strikes the analyzer 16, and is at least partially blocked from transmission to the lens 18. The invention does not require that the analyzer 16 have an axis of polarization which is perpendicular to the axis of polarization of the polarizer 12. It is possible to obtain an improved image of the culture medium 14 and bacterial colonies 24 with partial transmission of the reflected polarized light through the analyzer 16 which occurs when the polarization axes are not orthogonal. The analyzer may be held in a receptacle which permits rotary adjustment of the polarization axis relative to the polarization axis of the polarizer which is typically fixed. The non-polarized back-scattered light is merely plane polarized as it passes through the analyzer to the lens 18. This back-scattered light forms an image of reduced intensity compared to no polarizer being present and is without specular reflection, which has been discovered to be the source of degraded optical images of visible bacterial colonies resulting in erroneously high counts produced by automated colony counters.

In Fig. 1 the use of a present invention to read individual microbial colonies 24 is illustrated. When incubated on culture media, individual colonies produce a raised pattern on the otherwise flat surface of the culture medium 14. As illustrated therein, the polarized light reflected from the surface of individual bacterial colonies and the area surrounding the culture medium 14 is blocked by the analyzer 16. However, polarized light that strikes the visible bacterial colonies 24 and penetrates somewhat into the

individual colonies becomes non-polarized back-scattered light, which then passes through the analyzer 16, and is formed into a video image by the camera 22. The resultant image has reduced intensity in view of the analyzer 16 partially blocking the passage of the non-polarized back-scattered light and further has no specular reflection, which degrades the image quality and can interfere with the automated counting of the visible colonies. It should be understood that only a single colony 24 is illustrated for purposes of explaining the operation of the present invention but, in actual practice, as discussed below in conjunction with Figs. 4-7, typically numerous colonies are visible which may be automatically counted by software in a programmed PC (not illustrated) which is connected to and analyzes the video image produced by the camera 22.

In Fig. 2, a zone of inhibition 26, which is surrounded by bacterial colonies 24, is being read that takes the form of a circular area in which no bacterial growth occurs surrounded by continuous bacterial colonies. As illustrated the bacterial lawn is raised from the surface of the culture medium 14. The overall operation is similar to that described above with respect to Fig. 1 in that reflected polarized light transmitted from the bacterial lawn and the zone of inhibition is blocked by the analyzer 16, which results in a video image of only the back-scattered light being produced by the video camera 22.

Fig. 3 illustrates the use of the present invention for reading a SGE streak which is composed of bacterial colonies 24. The reading of Fig. 3 is similar to the operation of the present invention for reading bacterial colonies of Fig. 1 and a zone of inhibition of Fig. 2 with only back-scattered light from the SGE streak being transmitted through the analyzer 16 so that only a video image of back-scattered light on the culture medium 14 and the SGE streak is produced.

Fig. 4 illustrates a video image of an incubated culture medium produced by camera 22 in accordance with the present invention when the analyzer is opened, meaning that the axis of polarization is oriented to pass polarized light produced by the polarizer 12. In this circumstance, specular reflection is produced which degrades the image. Overall, the image has specular reflection which is a source of producing erroneously high colony counts as described below. The circular light-colored patterns are individual bacterial colonies 30 produced by the inoculation of a sample containing bacteria onto the culture medium for a preset period of time.

Fig. 5 illustrates a video image produced by the video camera 22 of the same incubated culture medium of Fig. 4 with the analyzer polarization oriented to block polarized light produced by the polarizer 12. The overall image has decreased brightness and an absence of reflections, which to the naked eye is somewhat difficult to see, but enhances the accuracy of automated colony counting as described below.

Fig. 6 illustrates a video image produced by the camera 22 of the same incubated culture medium of Fig. 4 after the resultant video image has been processed by automated colony counting software in a programmed PC, which forms no part of the present invention. That software produces the small circular markers 32 that are within the larger white bacterial colonies 30. The small circular markers 32 within the bacterial colonies 30 are placed by the automated colony counting software to identify each area which has been counted as a single colony. As is apparent, several of the large visible bacterial colonies 30 have numerous smaller circular markers 32 therein, which are an indication that the automated colony counting software has erroneously counted multiple bacterial colonies within the large individual colonies. The image of Fig. 6 was formed with the analyzer 16 oriented in the same position as Fig. 4.

Fig. 7 illustrates an image produced by the video camera 22 of the same incubated culture medium of Fig. 5 with the analyzer 16 polarization axis in the same orientation as in Fig. 5 As is apparent, the small circular markers 32 within the larger colonies 30 have been reduced substantially in number which indicates that the colony counting software has produced a lower number of colony counts than that produced by the image of Fig. 5, which is more accurate and more clearly corresponds with the count that would be produced by human counting. The lower colony count number, while not 100% accurate, as can be verified by one of the large colonies still having multiple colony counts 32, is a distinct improvement over Fig. 6. As is seen, the remaining large white colonies have only a single individual small circle 32. This indicates that those areas have been counted as only a single colony count, which is the correct way that these large bacterial colonies should have been counted.

Given the desirability of reducing human labor in laboratories in order to reduce the overall costs in health care services, it is seen that the present invention substantially enhances the accuracy of automated colony counting produced by automated colony counters. This improvement is the result of the discovery that

specular reflection from the culture medium, the area surrounding the culture medium, and individual or groups of bacterial colonies is undesirable. The desired image of bacterial colonies is produced by reducing or eliminating specular reflection when only or substantially only back-scattered light emanating from the culture medium and the bacterial colonies passes through the analyzer to the image-producing device.

While the present invention has been described in terms of its preferred embodiments, it should be understood that numerous modifications may be made thereto without departing from the spirit and scope of the present invention. It is intended that all such modifications fall within the scope of the appended claims.

CLAIMS

1. An apparatus for viewing a culture medium having visible bacterial colonies comprising:

a light source, which provides incident light on the culture medium having the visible bacterial colonies;

a polarizer, which polarizes the incident light;

an image-producing device having a field of view, including the culture medium, which provides an image of the field of view; and

an analyzer, which at least partially blocks reflected polarized light from being transmitted from the bacterial colonies and culture medium to the image producing device and which passes back-scattered light transmitted from the bacterial colonies and the culture medium to the image-producing device to cause the image-producing device to produce an image of the back-scattered light of the visible bacterial colonies and the culture medium.

2. An apparatus in accordance with claim 1 wherein:

the polarizer is disposed between the culture medium and the light source; and

the analyzer is disposed in the field of view between the image producing device and the culture medium.

3. An apparatus in accordance with claim 1 wherein:

the reflected light is from individual bacterial colonies.

4. An apparatus in accordance with claim 1 wherein:

the reflected light is from a transition of an area of growth of bacterial colonies to an area of no growth of bacterial colonies.

5. An apparatus in accordance with claim 2 wherein:

the reflected light is from individual bacterial colonies.

6. An apparatus in accordance with claim 2 wherein:

the reflected light is from a transition of an area of growth of bacterial colonies to an area of no growth of bacterial colonies.

7. An apparatus in accordance with claim 1 wherein:

the image producing device is a camera.

8. An apparatus in accordance with claim 1 wherein:

the polarizer and analyzer each linearly polarizes light and the analyzer has a polarization axis which is orthogonal to a polarization axis of the polarizer.

9. An apparatus in accordance with claim 2 wherein:

the polarizer and analyzer each linearly polarizes light and the analyzer has a polarization axis which can be orthogonal to a polarization axis of the polarizer.

10. An apparatus in accordance with claim 3 wherein:

the polarizer and analyzer each linearly polarizes light and the analyzer has a polarization axis which can be orthogonal to a polarization axis of the polarizer.

11. An apparatus in accordance with claim 4 wherein:

the polarizer and analyzer each linearly polarizes light and the analyzer has a polarization axis which can be orthogonal to a polarization axis of the polarizer.

12. An apparatus in accordance with claim 5 wherein:

the polarizer and analyzer each linearly polarizes light and the analyzer has a polarization axis which can be orthogonal to a polarization axis of the polarizer.

13. An apparatus in accordance with claim 6 wherein:

the polarizer and analyzer each linearly polarizes light and the analyzer has a polarization axis which can be orthogonal to a polarization axis of the polarizer.

14. An apparatus in accordance with claim 7 wherein:

the polarizer and analyzer each linearly polarizes light and the analyzer has a polarization axis which can be orthogonal to a polarization axis of the polarizer.

15. A method of viewing a culture medium having visible bacterial colonies with an apparatus having a light source, a polarizer, an image-producing device and an analyzer comprising:

illuminating the culture medium with light from the light source which passes through the polarizer before illuminating the culture medium with polarized light;

transmitting reflected and back-scattered light from the culture medium and the bacterial colonies on the culture medium to the analyzer, which at least partially blocks the polarized light from being transmitted to the image-producing device and transmits the back-scattered light to the image-producing device; and

producing an image with the back-scattered light of the bacterial colonies and the culture medium with the image-producing device.

16. A method in accordance with claim 15 wherein:

the reflected and back-scattered light is transmitted from individual bacterial colonies.

17. A method in accordance with claim 15 wherein:

the reflected and back-scattered light is from a transition of an area of growth of bacterial colonies to an area of no growth of bacterial colonies.

18. A method in accordance with claim 15 wherein:

the polarizer and analyzer each linearly polarizes light; and

the analyzer has a polarization axis which can be orthogonal to a polarization axis of the polarizer.

19. A method in accordance with claim 16 wherein:

the polarizer and analyzer each linearly polarizes light; and

the analyzer has a polarization axis which can be orthogonal to a polarization axis of the polarizer.

20. A method in accordance with claim 17 wherein:

the polarizer and analyzer each linearly polarizes light; and

the analyzer has a polarization axis which can be orthogonal to a polarization axis of the polarizer.

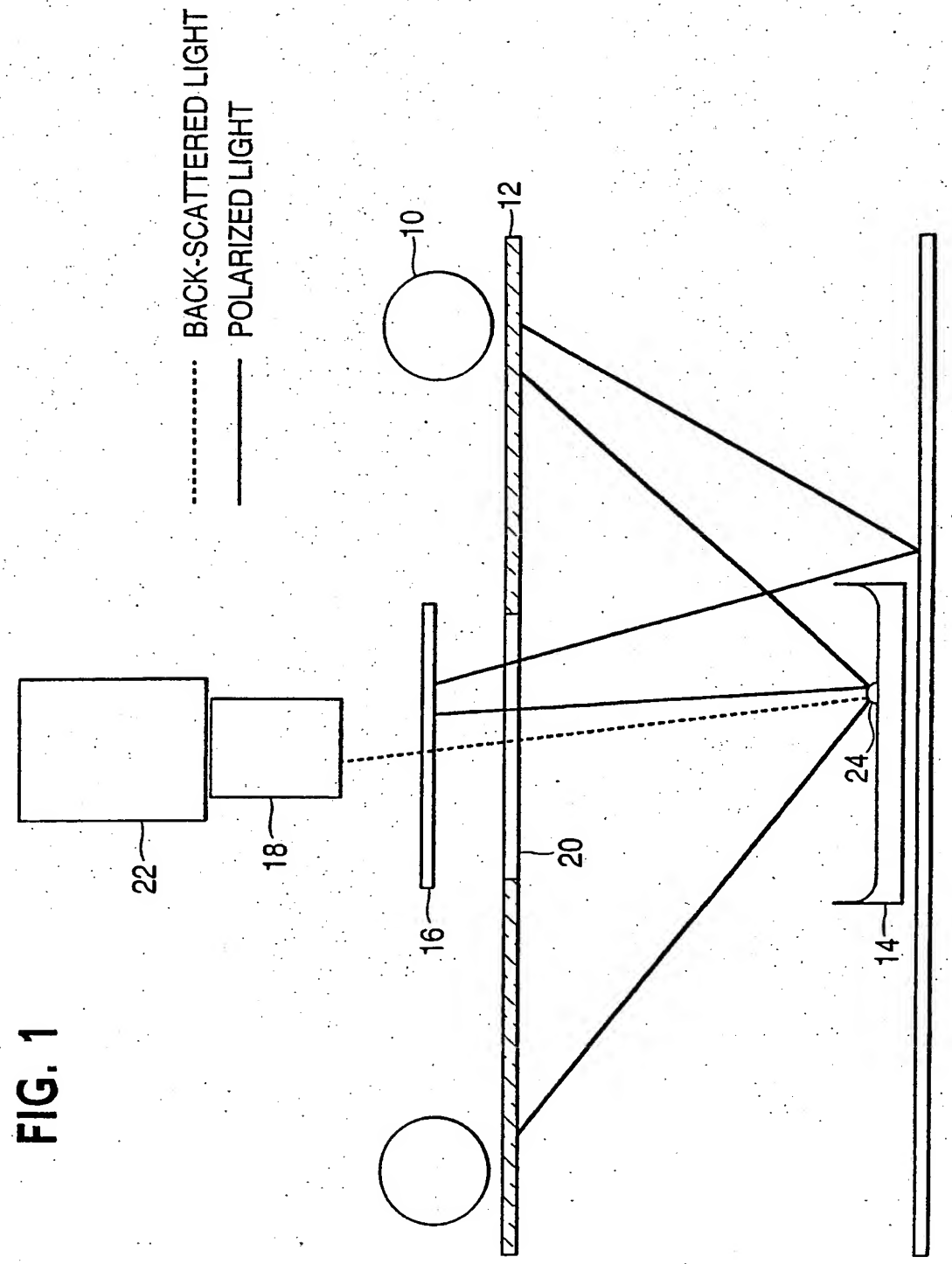


FIG. 1

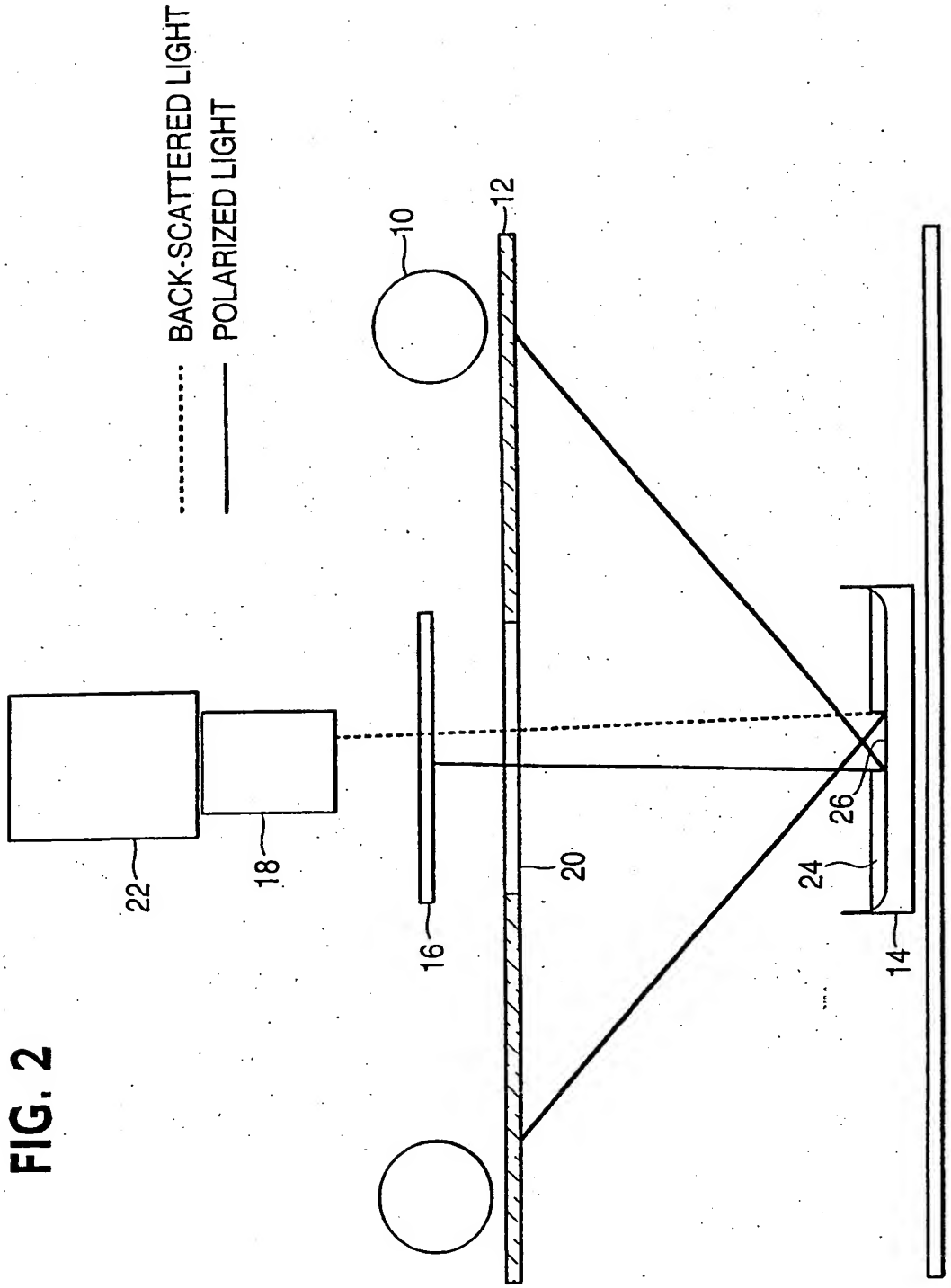

BACK-SCATTERED LIGHT
POLARIZED LIGHT
10
12
22
18
16
20
24
26
14

FIG. 2

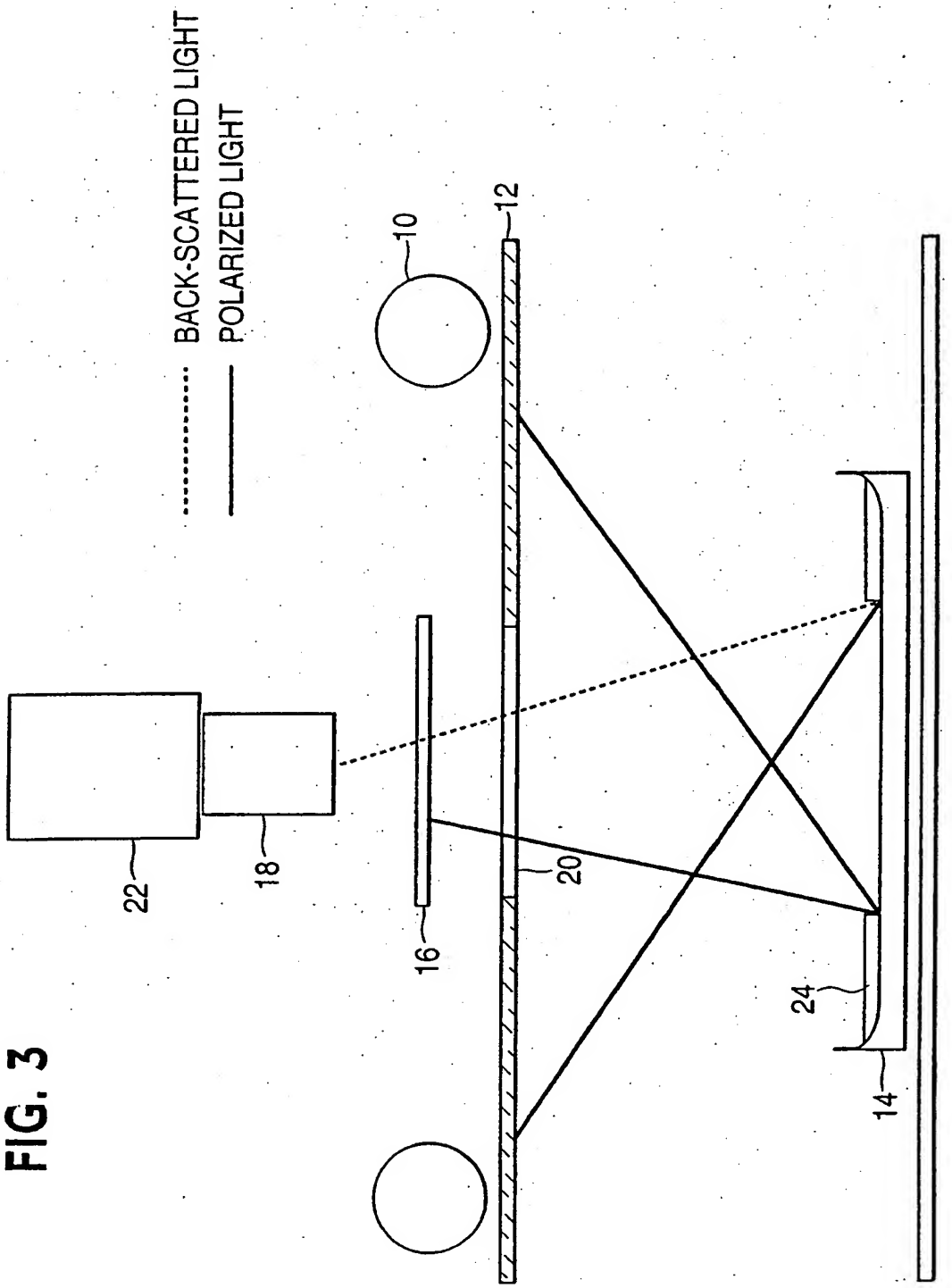


FIG. 3

FIG.4

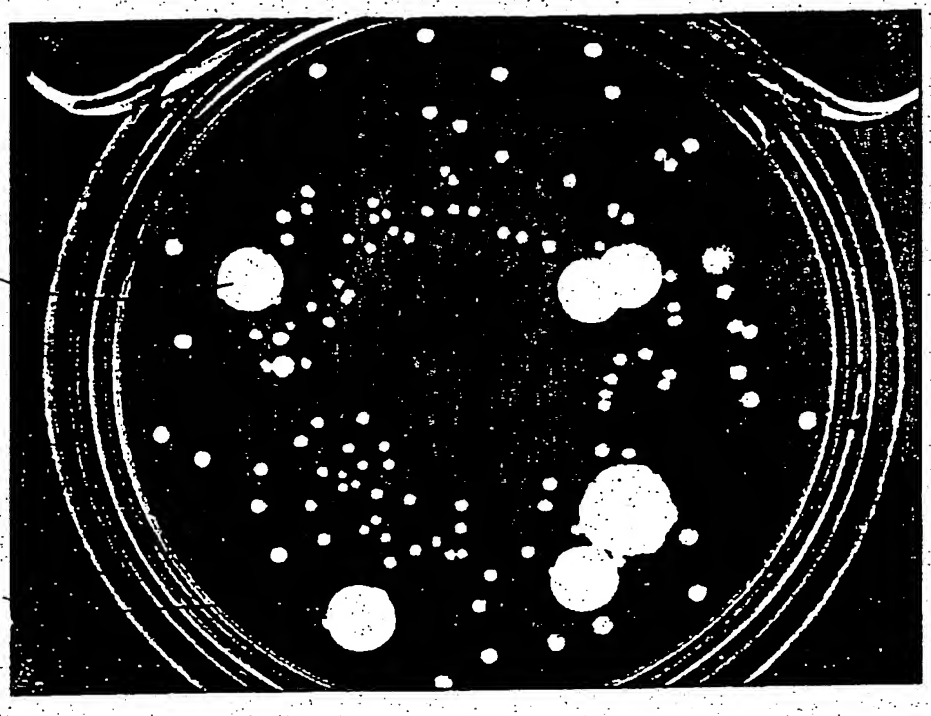


FIG.5

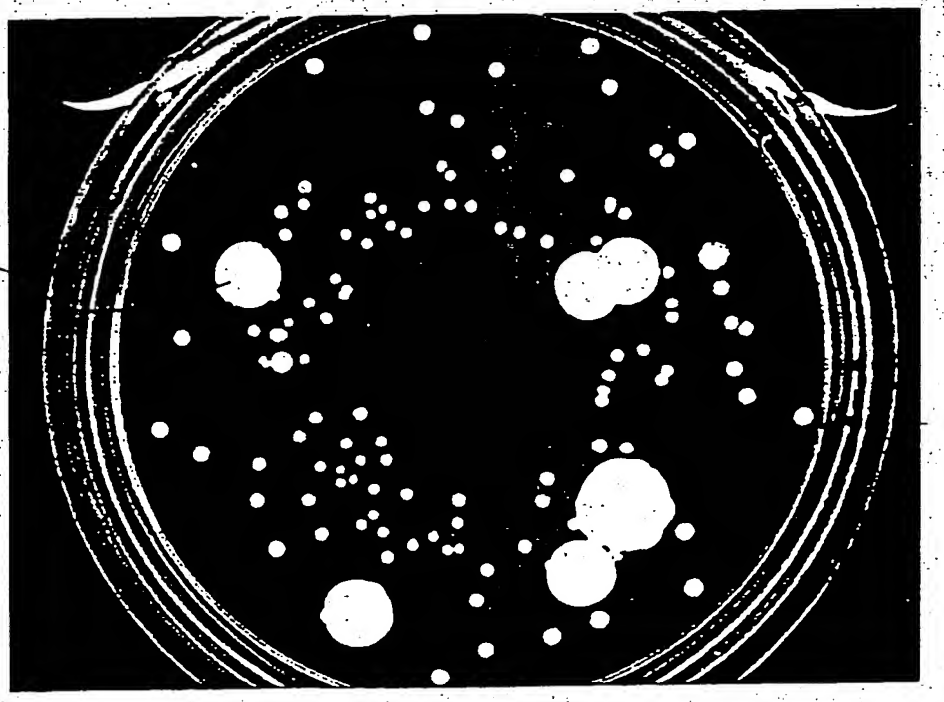


FIG. 6

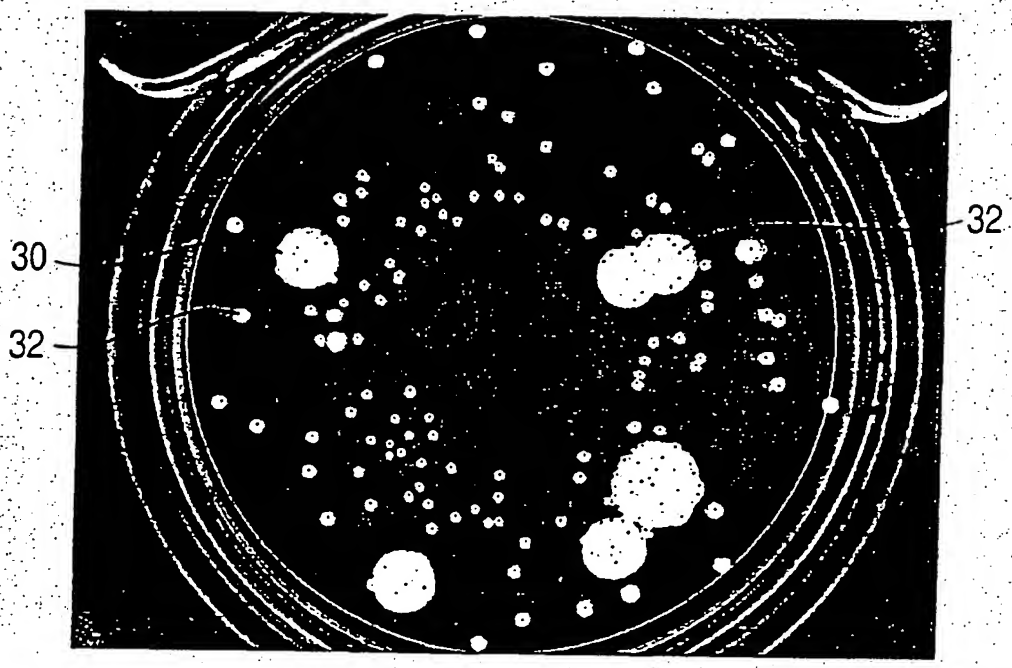
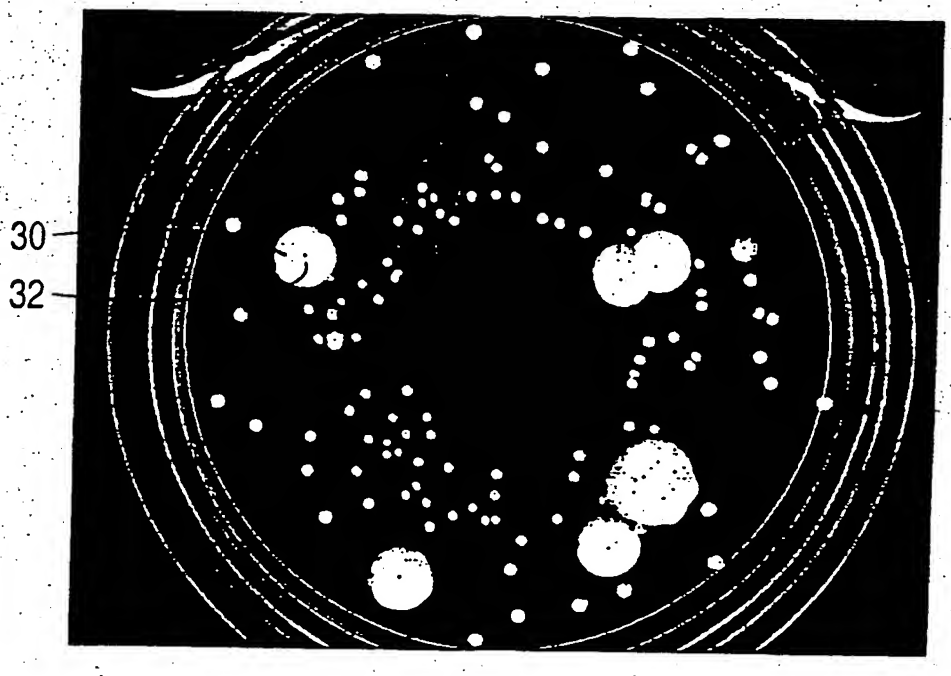


FIG. 7



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